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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/EP90/02015 <b>(22) International Filing Date:</b> 26 November 1990 (26.11.90) <b>(30) Priority data:</b> 22520 A/89 27 November 1989 (27.11.89) IT <b>(71)(72) Applicant and Inventor:</b> DIOGUARDI, Francesco, Saverio [IT/IT]; Via Ciovasso, 11, I-20121 Milan (IT). <b>(74) Agents:</b> GERVASI, Gemma et al.; Notarbartolo & Gervasi S.r.l., Viale Bianca Maria, 33, I-20122 Milan (IT). <b>(81) Designated States:</b> AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH (European patent), CM (OAPI patent), DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GA (OAPI patent), GB (European patent), GR (European patent), HU, IT (European patent), JP, KP, KR, LK, LU (European patent), MC, MG, ML (OAPI patent), MR (OAPI patent), MW, NL (European patent), NO, RO, SD, SE (European patent), SN (OAPI patent), SU, TD (OAPI patent), TG (OAPI patent), US.		<b>Published</b> <i>With international search report.</i>

**(54) Title:** USE OF GUANIDINOACETIC ACID TO INDUCE AN INCREASE OF THE CREATINE CONTENTS IN MUSCLES

**(57) Abstract**

Guanidinoacetic acid, or one of its salts, by itself or in association with methionine or with sulpho-adenosil-methionine (SAME) is utilized in the attachments and the physical conditions which require an increase of the intercellular muscular contents of creatine.

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USE OF GUANIDINOACETIC ACID TO INDUCE AN INCREASE OF THE CREATINE  
CONTENTS IN MUSCLES

Prior Art

It is known that in the muscular tissue exist different biological  
5 compounds of an energetic content of which the principal one is  
adenosine triphosphate (ATP).

Among these substances a very important one is creatine phosphate,  
which is in relationship with ATP in the sense that between the two  
compounds there is an exchange of phosphate groups.

10 The importance of creatine is demonstrated by the fact that in  
conditions of myocardial ischemia there is an arrest of the  
contractile function of the heart at the moment of the depletion of  
the creatine itself while 90% of the ATP is still present.

This is due to the fact that the ATP available to give immediate  
15 energy for the contraction is only a small part of the ATP present,  
which functions as a reserve. The duty of creatine is to recharge  
the ATP until it can give energy for the contraction.

From these considerations an important problem clearly emerges,  
that is, the problem of increasing, when necessary, the amount of  
20 creatine in the muscle. This would allow a prolonged efficiency and  
more potency of both the cardiac and skeleton muscle.

In fact, while the reserve of ATP is easily refurnished both in  
aerobiosis and in anaerobiosis, the contractile performance  
peculiarly in anaerobiosis, is a function of the quantity of  
25 creatine present.

Therefore, said substance is of great interest both in the medical and sports field: one only has to think, for example, of how many lives could be saved in case of myocardial infarct if it would be possible to make the cardiac contractions continue in conditions of ischemia until a correct oxygenation of the infarcted tissue is restored or, in the case of sports activity, how well an athlete could perform in a sport with such an explosive type of effort, as in the 100 meter race, where the athlete carries out his performance in apnea.

10 On the other hand, it should be kept in mind that creatine is synthesized principally in the kidneys and in the liver and therefore, there will be a deficiency of it in the course of all the nephropathies and hepatopathies with a parenchymal derangement. Unfortunately, the administration of exogenous creatine does not

15 bring any positive result, because exogenous creatine inhibits the synthesis of endogenous creatine for a quantity equal to the quantity of creatine administered.

#### Summary

We have now found that the prior art difficulties can be overcome

20 through the administration of guanidinoacetic acid or of one of its salts, alone or in association with methionine or with sulpho-adenosyl-methionine (SAmE).

Such an administration brings an increase of the intercellular muscular content of creatine and therefore increases the

25 availability of energy for both the skeletal and cardiacal muscular

cells.

Detailed description of the invention

The effects and the advantages of the administration of guanidinoacetic acid (GAA) alone or in association with methionine  
5 or sulpho-adenosyl-methionine (SAME) in the aim of increasing the intercellular muscular content of creatine will be further illustrated in the course of the following detailed description.

In an experiment conducted with rats GAA was administered as a supplement to a standard diet. To determine the increase of the  
10 intercellular muscular content of creatine, the urine excreted by them during 24 hours was collected and the concentration of creatinine was measured. The dosage provides a reliable measure of the intercellular content of creatine in that, as known, the renal elimination of creatinine is a mathematical function of the  
15 intercellular concentration of creatine and does not depend on any other parameter as, for example, muscular exercise or calorie intake.

The experimentation with GAA was realized in two stages each of a week's duration, and intervalled by a period of three days between  
20 the first and second stage. In the week preceeding the experimentation with GAA the creatininuria was monitored in the rats fed with a diet which contained methionine in an amount equal to the one needed by the rat plus an amount of methionine of 1.7 mg/kg of body weight, which corresponds to the one necessary to  
25 activate the GAA which will be added in the successive weeks.

In the first stage of the experiment with GAA the rats were fed with the diet of the preceding week supplemented with GAA in quantity of 1.7 mg/kg of body weight.

After a week, the integration of the diet with GAA was suspended for three days. After this suspension the rats were fed with a diet supplemented with GAA again for one week (second stage).

The results obtained are reported in Table 1.

TABLE 1: Creatininuria (mg/24 h) in rats fed with a diet supplemented with GAA

10 (average values of a week  $\pm$  standard deviation)

Before the treatment	After treatment 1st stage	After treatment 2nd stage
5.2 $\pm$ 0.1	7.8 $\pm$ 0.5	9.2 $\pm$ 0.3

It should be observed that in the synthesis process of creatine starting from GAA there is the danger of subtracting methionine from other metabolic processes. For this reason the present invention also foresees the association of methionine or SAME to GAA with a molar ratio between methionine or SAME and GAA of between 0.5:1 and 3:1.

20 The administration of GAA and of the association GAA-methionine or GAA-SAME therefore finds a very important indication in all the conditions in which it is necessary or opportune to increase the intramuscular concentration of creatine and particularly in the nephropathies and hepatopathies with parenchymal damage, in older people, in conditions of hyponutrition and chronic and acute

myocardial muscle ischemia.

Such substances are also usefully administered to athletes who practice sports which require explosive efforts. The administration of GAA and of the GAA-methionine or GAA-SAMe association can be  
5 made orally or parenterally employing said substances in a pure state or in the form of compositions comprising diluents and pharmacologically acceptable excipients. GAA can be used as such or in the form of salt with a pharmacologically acceptable cation.

The daily dose of GAA to be administered is comprised between  
10 0.0001 to 5 mg/kg of body weight in several daily doses.

## CLAIMS

- 1 1. Use of guanidinoacetic acid or of one of its salts for  
2 administration in affections and physical conditions which require  
3 an increase of the intracellular muscular contents of creatine.
- 1 2. Use according to claim 1, characterized in that the  
2 guanidinoacetic acid or one of its salts is in association with  
3 methionine or with SAMe in a molar ratio between methionine or SAMe  
4 and guanidinoacetic acid comprised between 0.5:1 and 3:1.
- 1 3. Use of guanidinoacetic acid or one of its salts for the  
2 preparation of pharmaceutical compositions comprising diluents and  
3 pharmacologically acceptable excipients suitable for the treatment  
4 of affections and physical conditions which require an increment of  
5 the intracellular muscular contents of creatine.
- 1 4. The use according to claim 3, characterized in that the  
2 guanidinoacetic acid or one of its salts is in association with  
3 methionine or with SAMe in a molar ratio between methionine or SAMe  
4 and guanidinoacetic acid comprised between 0.5/1 and 3:1.
- 1 5. The use according to claims 1 to 4, characterized in that the  
2 daily quantity of guanidinoacetic acid to be administered is  
3 comprised between 0.0001 and 5 mg/kg of body weight in several  
4 daily administrations.

## INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 90/02015

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (If several classification symbols apply, indicate all) <sup>6</sup>		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC <sup>5</sup> : A 61 K 31/195, A 61 K 31/70		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
IPC <sup>5</sup>	A 61 K	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>8</sup>		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>9</sup></b>		
Category <sup>10</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
X	Adv. Enzymol., vol. 50, 1979, J.B. Walker: "Creatine: biosynthesis, regulation, and function", pages 177-242, see page 194, paragraph d: "Effects of Dietary Precursors of Creatine on Creatine Biosynthesis"	3
X	Ann. New York Acad. Sci., vol. 494, 1985, B. Cohen et al.: "Methionine and the control of creatine synthesis", pages 329-331, see the whole article	4
A	Archives of Biochemistry and Biophysics, vol. 220, no. 2, 1 February 1983, Academic Press, Inc., J.J. Roberts et al.: "Synthesis and	3, 4
<p>* Special categories of cited documents: <sup>14</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
31st January 1991	12.03.91	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	Alfredo Prein	

## FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

accumulation of an extremely stable high-energy phosphate compound by muscle, heart, and brain of animals fed the creatine analog, 1-carboxy-ethyl-2-iminoimidazolidine (homocycIo-creatine)",  
pages 563-571,  
see the whole article

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/incompletely

V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND ~~UN~~SEARCHABLE

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☒ Claim numbers XX, because they relate to subject matter not required to be searched by this Authority, namely:

xx Claims 1,2,5 are not searched

Pls. see Rule 39.1 (IV) - PCT:

Method for treatment of the human or animal body by surgery or therapy as well as diagnostic methods.

2. ☒ Claim numbers XX, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

xx Claim 3 searched incompletely

Reason: It is not completely clear which diseases are meant by: "... affections .... creatine."

3. ☐ Claim numbers \_\_\_\_\_, because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the international searching authority did not invite payment of any additional fee.

## Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.